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Mutational Analysis of AREA, a Transcriptional Activator Mediating Nitrogen Metabolite Repression in *Aspergillus nidulans* and a Member of the "Streetwise" GATA Family of Transcription Factors

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INTRODUCTION

A major family of transcription factors, the GATA proteins, are distinguished by their distinctive Cys_2-Cys_2 zinc finger- and adjacent basic region-containing DNA binding domain (DBD) which recognizes the core consensus 5′-GATA (34, 35, 44, 45, 51, 52, 55, 79). ("Gata" means street in Old Norse and, for example, modern Swedish. Hence, GATA family transcription factors can be said to know their way around the streets, i.e., be "streetwise.") GATA family transcription factors occur very widely among eukaryotes. At least six members playing a variety of essential roles have been identified in vertebrates (reviewed in references 70 and 81), while other members have been identified in, for example, *Nicotiana* (30), *Drosophila* (1, 48, 61, 66), *Bombyx* (32, 33), *Caenorhabditis* (39, 73), and *Dictyostelium* (25) species. Within the fungi examples of GATA family members include four involved in nitrogen regulation (26, 72, 74) and one involved in mating-type switching and pseudohyphal growth (21, 24a, 69) in *Saccharomyces*, one involved in iron acquisition in *Ustilago* (80), and two involved in photoresponses and circadian regulation in *Neurospora* (19a, 20, 28, 49).

The *areA* gene of the ascomycete fungus *Aspergillus nidulans* encodes a GATA family transcriptional activator (AREA) mediating nitrogen metabolite repression, allowing preferential utilization of the most favorable nitrogen sources ammonium and L-glutamine (7, 23, 27, 42, 45, 46, 51, 83). Like most other fungal GATA factors, AREA contains only a single copy of the distinctive zinc finger-containing region of GATA factor homology in its 876 residues, whereas vertebrate GATA factors contain two copies of somewhat divergent sequence. The AREA DBD more closely resembles the C-terminal GATA homology region of vertebrate GATA factors, which in some, but not all, cases plays the lion's role in DNA binding (50a, 52, 55, 57, 81a, 90). The greater C-terminal copy similarity to AREA is also true for a two-GATA region protein from *Ustilago* where the C-terminal GATA motif apparently plays the greater role in DNA binding (4, 80). As in most other GATA factors, the zinc finger of AREA has a 17-residue loop, whereas a few GATA factors, reported thus far only for fungi and a plant, contain an 18-residue loop (summarized in references 49 and 77a), although a mutant form of AREA containing an 18-residue loop is functional (see below). The nutritional versatility of *A. nidulans*, the ease with which nutrient utilization can be monitored in plate tests, and the amenability of the organism to both classical and reverse genetic manipu-

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lations allow assessment of the phenotypes of large numbers of mutations, and indeed, numerous *areA* mutations have been described, many of which have been characterized at the sequence level (2, 3, 7, 16, 17, 22, 42, 46, 59, 60, 62, 63, 75). The task of sequencing *areA* mutations is much reduced by the facility of fine-structure mapping (5), which also allows the combining or separating of mutations (22, 59).

Wide variations in interactions between AREA and the numerous AREA-controlled promoters result in a startling heterogeneity of *areA* mutant phenotypes. This heterogeneity and its nonhierarchical nature led to the prediction that the *areA* gene product directly regulates gene expression long before *areA* had been cloned (6, 7, 15, 83). Thus, a given *areA* mutation might lead to 20% of wild-type expression of structural gene A, 80% expression of structural gene B, 250% expression of structural gene C, and no expression of structural gene D, whereas another *areA* mutation might lead to 70% expression of A, no expression of B, 10% expression of C, and wild-type expression of D.

The ability of the AREA DBD to bind specifically to GATA sequences is well documented (24, 46, 52, 58, 60, 64, 65, 76, 77). GATA sequences in at least two promoters have been shown to be physiologically relevant to AREA control (46, 65). On the basis of current data, the sequence of physiologically relevant AREA binding sites is HGATAR (65).

The solution structures of complexes containing the wild type or specificity mutant (see below) L683V and cognate DNA sites have been determined (76, 77). The global fold of the wild-type AREA zinc finger core is very similar to that described by Omichinski et al. (55) for chicken GATA-1 (cGATA-1) with two antiparallel irregular β sheets composed of β-strands 1 (residues 671 to 673) and 2 (residues 677 to 680) and β-strands 3 (residues 684 to 687) and 4 (residues 690 to 693), respectively, connected by an extended loop and followed by an alpha-helical region (residues 694 to 704 terminated by helix-capping Gly-705) and an extended loop (residues 706 to 717, including a helical turn [residues 710 to 712]). The core module utilizes residues on one face of the alpha-helix and on the loop between the β_2 and β_3 strands to recognize the major groove of the DNA site. Compared to cGATA-1, AREA makes fewer minor-groove contacts because the basic C-terminal tail of the DBD, rather than lying in the minor groove, runs parallel to the sugar phosphate backbone along the edge of the minor groove (76). This correlates with reduced DNA binding affinity of AREA compared to cGATA-1 (76). The L683V substitution, which results in a clear preference for TGATA over CGATA and AGATA sites in contrast to the wild type, slightly shifts the AREA backbone in the vicinity of the mutant valine residue, the changing hydrophobic packing interactions probably accounting for the change in specificity (65, 77).

AREA provides a potential model for analysis of the DBDs of other members of the ever-growing GATA family of transcription factors. Here we survey and, where possible, annotate 109 mutant sequences in the AREA DBD, including both functional and nonfunctional mutant AREA forms, and the results of both classical mutagenesis and site-directed mutagenesis. Certain sequence changes in the AREA DBD lead to nitrogen metabolite-derepressed expression of AREA-controlled structural genes (59). As the basis for this derepression is almost certainly the prevention of a protein-protein interaction (4a, 56, 59, 87) and as different members of the GATA family are apparently involved in a wide variety of different protein-protein interactions (reviewed in reference 50), the derepression is unlikely to form the basis for generalizations throughout the GATA family and will therefore not be discussed here.

A small number of mutational changes in the DBDs of other GATA factors have been described. Relevant comparisons will be noted here. Especially notable are site-directed mutagenic changes (36, 56, 88) in the DBD of *Neurospora crassa* NIT2 (reviewed in reference 51), an isofunctional homolog of AREA. However, caution is necessary in comparing the NIT2 data with those of AREA for three reasons. Firstly, a relatively high proportion of the NIT2 mutant changes involve double or even triple substitutions, complicating interpretations. Secondly, NIT2 constructs were inserted ectopically and, even in the most recent study (56) where they were targeted to the *his-3* locus, there is no guarantee that mutant and wild-type NIT2 protein levels would be equivalent to those of proteins transcribed and translated from the resident *nit-2* gene (i.e., the physiologically relevant comparisons). Reduced protein levels could make a functional but impaired form of NIT2 appear to lack function, whereas elevated protein levels could give a deceptive impression of functionality for a substantially impaired form. The effects of overexpression on protein-protein interactions are another source of concern (82). Thirdly, the *N. crassa* transformants have apparently been tested for NIT2 functionality on only a few nitrogen sources, raising the possibility that mutant substitutions classified as nonfunctional might have been classified as functional had transformants been selected (in the untargeted work) or classified (when targeted to *his-3*) by using different nitrogen nutrients.

POWER OF SIMPLE PHYSIOLOGICAL ANALYSIS WITH *A. NIDULANS* AND CHOICE OF AREA

A. nidulans is a fast-growing, differentiating, filamentous fungus with a colonial growth habit and a voracious appetite for an astonishing range of nutrients. (Some years ago J.-M. Wiame remarked to one of us [H.N.A.] that, whereas *Saccharomyces cerevisiae* is a "gourmet," *A. nidulans* is a "gourmand.") Careful observation of mutant growth characteristics under a variety of different growth conditions on solid media can yield considerable information, a richer array of information that can normally be gleaned from nutritional tests with unicellular organisms or organisms having less nutritional versatility. The morphological consequences of lack of growth through nutrient starvation are, for example, usually very different from those resulting from toxicity of an exogenously supplied or endogenously generated inhibitor, and growth correlates closely with nutrient limitation or inhibitor toxicity. Mutations in the four-gene cluster involved in L-proline catabolism provide a good example. In a genetic background in which exogenous proline is both required and toxic, seven different classes of mutation, three single-gene classes plus four categories of deletions covering two or more genes, were identified with only eight media (8), a test combination which will also suffice, in an otherwise wild-type background, to distinguish virtually every class of single- and multiple-gene lesions (8, 11, 12, 43).

Proline catabolism also provides an illustration of the sensitivity with which subtle differences in growth can distinguish mutations likely to differ at the sequence level. More than two decades ago, proline-specific, extragenic *cis*-acting suppressors of *areA* loss-of-function mutations were selected and proposed to alleviate (partially) the requirement for AREA in proline catabolism by leading to carbon catabolite derepression (7, 11). Two such suppressors were found to differ slightly in the degree to which they restore the ability of *areA* loss-of-function mutants to utilize proline (12). Much later molecular analysis confirmed that these mutations destroy binding sites for the carbon catabolite repressor CREA and showed that the weak-

er and stronger suppressors destroy different (but nearly adjacent) CREA binding sites (29, 71). The degree of carbon catabolite derepression, as determined from steady-state transcript levels, closely parallels the degree of *areA* mutation suppression seen in growth tests (71).

AREA is particularly useful as a model GATA factor because of the ease with which its (dispensable) function can be observed and manipulated. Wild-type strains of *A. nidulans* utilize an extremely wide variety of nitrogen sources, including most of the L-amino acids (including some such as α-aminobutyrate and homoserine which are not normal protein constituents), a number of ω-amino acids, various amines, imines, imides, and amides (including lactams), some aminoalcohols, various purines and their degradation intermediates, taurine, hypotaurine, nitrate, and nitrite. Utilization of virtually all of these involves structural genes subject to AREA control. Additionally, *areA* mutations affecting the specificity of structural-gene expression can enable utilization of nitrogenous compounds such as acrylamide, L-histidine, L-citrulline, and 6-methoxypurine (7, 41, 42, 65, 67). Hence the effects of *areA* mutations on expression of numerous (>100) structural genes can be monitored by solid-medium nutritional tests, albeit not always by effects on individual genes. Given both the sensitivity with which growth differences can be detected on any one nitrogen source and the ability to monitor utilization of numerous nitrogen sources, distinguishing between even very subtly different mutant forms of AREA is frequently possible. Indeed, the limiting factor in making such distinctions might be the patience of the observer rather than the capabilities of nutritional testing.

The manipulability of AREA benefits from intragenic meiotic recombination (5) which allows, inter alia, the localization of mutations with considerable precision. (We have observed recombination within intervals as short as 12 bp.) In many cases this allows component mutations from multiply mutant alleles to be separated. By using crosses with suitably positioned *areA* loss-of-function mutations in repulsion, mutations other than those leading to loss of function can be combined. For example, a zinc finger loop mutational change altering specificity of GATA site recognition was recombined into the N-terminal zinc finger-encoding region of a tandem duplication mutant allele containing two copies of the DBD-encoding region (22) (see below).

Finally, the availability of *areA* loss-of-function mutations located throughout the gene greatly facilitates the insertion, by (partial) gene replacement, of site-directed *areA* mutations other than those leading to loss of function (46, 59, 60, 65). The use of PCR products containing a relatively small portion of *areA* avoids the problem of ectopic integration except where it occurs fortuitously.

DEFINING THE LIMITS OF THE AREA DBD

The AREA protein has been monitored for redundancy, both by deletion analysis and through the use of compensating frameshift mutations (16, 45, 46, 60, 75). Substitution of a normal sequence by one translated in another reading frame has the advantage over deletion analysis of preserving the primary-structure spatial relationship between domains but the potential disadvantage that out-of-frame translation sequences can have adverse (or, less likely, favorable) effects on protein function (45, 54, 60). In the case of AREA this analysis has led to the conclusion that there is considerable redundancy because large portions can be deleted or read in the wrong reading frame with only modest impairment of function. It thus seems likely that the only entirely indispensable region of AREA is the DBD, the only region where significant numbers of single-residue substitutions abolish function.

Delineation Based on Homology

A 52-residue region of AREA with striking similarity in ungapped alignment to the zinc finger-containing region of murine GATA-1 (9) constituted the first indication of the limits of the DBD as well as the first evidence for a GATA factor family extending across eukaryotic kingdoms. This region, residues 671 to 722, begins 2 residues before the first of the four zinc-chelating residues and ends 25 residues after the fourth, taking in a very basic region. It includes all of the residues which appear to be important in the DNA binding complex (76, 77). Although this homology-defined domain encompasses all of the extant nonfunctional residue substitutions in the vicinity, it fails to encompass two second-site suppressor substitutions (see below). Of course, residue substitutions can potentially create, as well as abolish, DNA contacts.

Delineation Based on Mutational Truncation

If it is assumed that the entire DBD, but no residue beyond it, is required for AREA function, then the boundaries between sequence truncations in which function is retained and sequence truncations in which function is lost define the limits of the DBD. At the C-terminal end two functional truncations are relevant. One ends the normal sequence with Asn-723 followed by a single out-of-frame encoded Gln residue (60, 75), and the other stops immediately after Arg-722 and thus includes no residues C-terminal to the region of GATA factor homology. In contrast, mutations creating a stop codon replacing that for Val-716 and a frameshift resulting in an out-of-frame translation product replacing Asn-721 and subsequent residues abolish function (75), suggesting that most, probably all, of the C-terminal moiety of the homology-defined DBD is necessary for function. Nevertheless, replacement of Arg-722 by Leu does not fully impede function (59).

Truncations relevant to the N-terminal limit of the DBD are provided by reversion of a frameshift mutation in codon 666. Revertants in which Glu-666 along with residues as far upstream as Ser-550 are replaced by an out-of-frame translation peptide appear fully functional, as do single-residue replacements of Glu-666 by Arg or Lys (84). Thus, (wild-type) residues encoded upstream of Gln-667 must not play a crucial role in DNA binding. At most, four residues N-terminal to the homology-defined DBD are necessary for AREA function.

MUTATIONAL ANALYSIS OF THE AREA DBD

Loss-of-Function Changes

Figure 1 shows the wild-type AREA sequence in the region of GATA factor homology and its environs, contains outline structural features and contacts according to Starich et al. (76), and indicates single-residue substitutions and single- or contiguous-residue deletions which abolish AREA function (resulting in inability to utilize nitrogen sources other than ammonium and L-glutamine). In some cases the basis for the loss-of-function phenotype is quite obvious; for example, changes involving the zinc-chelating cysteine residues or changes removing or introducing a proline residue, which are likely to have major structural implications. In addition, nonconservative changes in Pro-682, Arg-685, Asn-695, Ala-696, Leu-699, and Arg-708 (Fig. 1) could all impair important major-groove contacts (76). A696P would presumably break the alpha-helix. Thr-678, Thr-681, Pro-682, Trp-684, and Ala-696 are involved

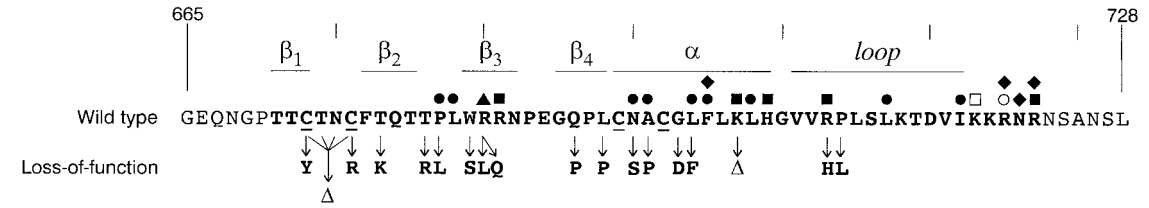

FIG. 1. Extant loss-of-function single-residue substitutions and single- or contiguous-residue deletions within residues 665 to 728 (numbered as in reference 46 and EMBL entry X52491) of AREA (2, 19, 42, 45, 59, 60, 62, 63). The region of GATA factor homology is boldfaced. Zinc-chelating Cys residues are underlined. Short raised vertical lines indicate residues 675, 685, 695, 705, 715, and 725. Raised horizontal lines indicate the extents of β-strands 1 to 4, the alpha-helix, and the extended loop (76). Residues involved in DNA contacts (76) are indicated as follows: major groove, filled symbols; minor groove, open symbols; hydrophobic interactions, circles; electrostatic interactions, squares; hydrogen bonds, triangles; interactions involving backbone amide or alpha protons, diamonds. Single- or contiguous-residue substitutions or deletions (Δ) resulting in loss of AREA function are indicated below the sequence. The double-headed arrow from Arg-685 indicates that both R685L and R685Q substitutions abolish function. The quadruple-tailed arrow from residues 673 to 676 indicates that deletion of these four adjacent residues leads to loss of function. Certain of these sequence changes have been obtained in strains carrying other *areA* mutations from which they have not been separated and which might influence their phenotypes: C673R, R685L, and N695S in an *areA1601* (75) background; Q691P and G698D in an L683V (7, 42, 45) background; and L693P in a ΔKTD713-715 (52) background. A696P results in loss of function in both wild-type (Leu-683) and L683V backgrounds (59).

in important DBD-stabilizing hydrophobic interactions (76) susceptible to disruption by substitutions. Deletion of Lys-702 (Fig. 1) would eliminate two major-groove phosphate contacts (76) as well as shorten the alpha-helix. Changes corresponding to P682L, R685L, and G698D in the *Ustilago maydis* iron acquisition GATA factor Urbs1 also result in loss of function (4). Changes in the Trp residue corresponding to Trp-684→Glu, →Met, or →Val in the isofunctional *N. crassa* homolog NIT2 abolish function (88).

Functional Substitutions or Deletions

Figure 2 shows extant single-residue substitutions and single- or contiguous-residue deletions compatible with retention of some AREA function (i.e., allowing significantly better utilization of at least one nitrogen source than the product of a null *areA* allele) both in an otherwise wild-type background (Fig. 2A) and in an L683V specificity change background (Fig. 2B). These substitutions and deletions have been obtained through forward selection of mutations using positive isolation procedures, reversion of loss-of-function mutations, site-directed mutagenesis followed by gene replacement, fine-structure recombination, or a combination of these. Those present in an L683V background are numerous because a number of loss-of-function mutations have been selected in this background and because of the extensive characterization of the L683V change (7, 14, 22, 31, 37, 41, 42, 45, 65, 77).

All the changes from Leu-683 (Fig. 2A), except possibly L683N, have phenotypes indicating that they alter the specificity of structural-gene activation (14, 42, 65), and the phenotype of ΔKTD713-715 is equally striking in that it does not noticeably affect utilization of some nitrogen sources such as nitrate, nitrite, and L-arginine but virtually abolishes utilization of others such as hypoxanthine and L-aspartate (59, 78). Acceptable substitutions and deletions for residues involved in DNA contacts in addition to those involving Leu-683 include A696S, L699C, L703F, L703V, L703S, K718E, K718L, K718Q, and R722L in a wild-type background (Fig. 2A) and P682S, A696S, L703F, L703V, L703S, and ΔL703 in an L683V background (Fig. 2B). In these cases, therefore, either the contacts involved are not essential and/or the acceptable substitutions or deletion allows alternative contacts involving the replacement residues or their neighbors. Changes in *U. maydis* Urbs1 corresponding to P709S and changes in *N. crassa* NIT2 corresponding to L683M, L683A, and L683V as well as those equivalent to substitution by Glu at position 683, by Met or Gln at position 701, and by Gly, Glu, or Lys at position 707 all allow retention of function (4, 56, 88).

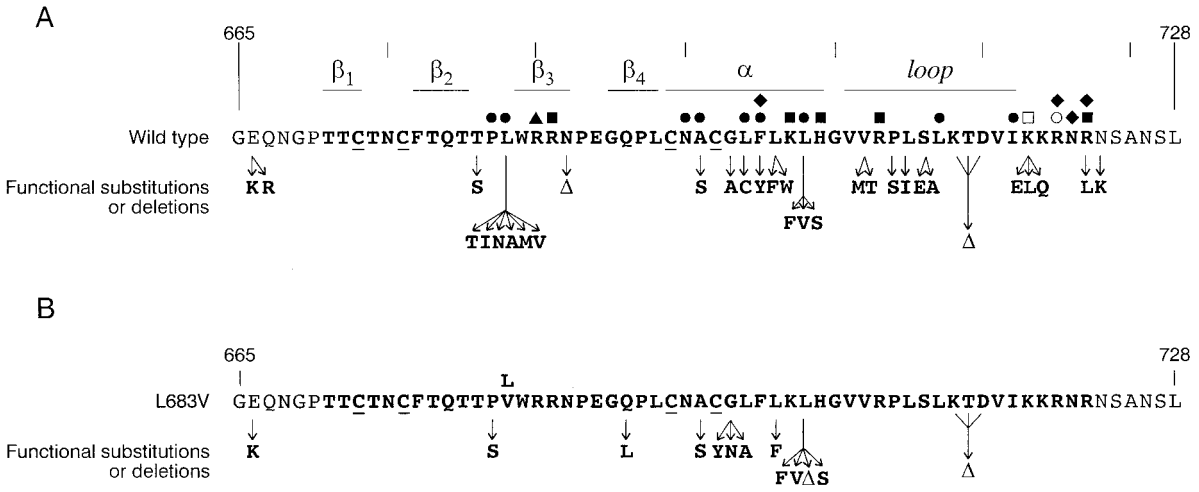

FIG. 2. (A) Extant functional single-residue substitutions and single- or contiguous-residue deletions within residues 665 to 728 of AREA (45, 59, 65, 86). Notation and structural information are in the legend to Fig. 1. (B) Extant functional substitutions and deletions in an L683V background (59, 85). The sequence includes the mutant Val residue at position 683 with the wild-type Leu residue shown above.

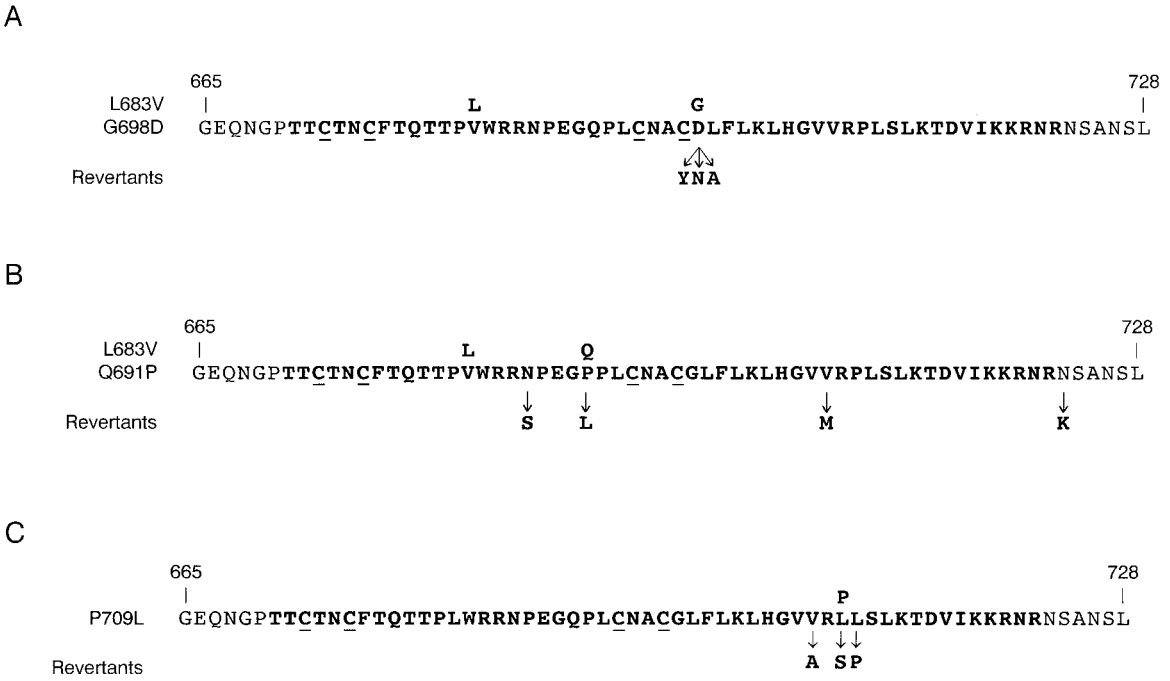

FIG. 3. (A) Revertants of G698D in an L683V background (10): (B) revertants of Q691P in an L683V background (10); (C) revertants of P709L (59, 62). Notation follows that for Fig. 2B.

An interesting question arises in conjunction with the specificity changes at position 683. Given that the L683V change (and presumably also the L683T, L683A, and to a lesser extent, L683I changes) increases binding to TGATA sites (65, 77) and assuming that this is what enables strains having this mutational change to utilize certain naturally occurring nitrogen sources not utilized by the wild type such as L-histidine (65), why do the presumed TGATA sites in the promoters concerned not simply accumulate mutations converting them to CGATA or AGATA sites, thus enabling the wild-type (Leu-683) organism to utilize these additional nitrogen sources? A possible answer is implicit in the discussion by Ravagnani et al. (65). They suggest that these promoters are controlled by pathway-specific transcription factors whose effector molecules are metabolically unrelated to the pathway in question and that the wild type appears unable to utilize these nitrogen sources because we do not know what coinducer to add and therefore no induction of the synthesis of the required enzyme(s) or permease occurs. The L683V change, by increasing AREA binding to TGATA sites in the promoter, then bypasses the requirement for induction and the pathway-specific regulator. If this hypothesis is correct, the replacement of TGATA sites by (A/C)GATA sites in the promoters concerned might also bypass the requirement for pathway-specific induction and result in unregulated, high-level expression of structural genes involved in histidine, etc., utilization. As histidine, etc., probably does not constitute a major and frequent source of nitrogen in nature, this lack of regulation would be disadvantageous.

A general observation on the changes shown in Fig. 2 is that the AREA DBD is remarkably tolerant to substitution and even deletion at a number of positions. This tolerance is of potential relevance to the evolution of the GATA factor family.

Reversion Spectra of Some Loss-of-Function Alleles

Reversion of most of the loss-of-function mutations in the *areA* DBD-encoding region has been attempted. In some cases either no revertants or only revertants with wild-type-derived protein sequence have been obtained. In a significant number of cases frameshift reversion has yielded an altered protein sequence and missense reversion has replaced the unacceptable mutant residue with an acceptable but mutant alternative or, in some instances, second-site suppressor substitutions for neighboring residues. Loss-of-function alleles giving rise to more than one mutant revertant form of AREA are discussed individually below, but we can note that T681S and L699C (Fig. 2A) were derived by reversion from T681R and L699F (Fig. 1), respectively, and that L701W (Fig. 2A) was derived by reversion from a −1 frameshift in codon 701.

G698D reversion. The loss-of-function substitution G698D is available only in an L683V background (42). No second-site suppressors were recovered, but revertants in which the unacceptable Asp residue was replaced by Ala, Asn, or Tyr have been obtained (Fig. 3A). A possibly surprising finding is that the Tyr substitution appears much more functional than the Asn substitution, virtually irrespective of the nitrogen nutrient. (The Ala substitution appears fully functional, which is unsurprising since it replaces the wild-type Gly.)

A696P reversion. The loss-of-function substitution A696P was originally obtained in an L683V background (42), but the serendipitous recovery of a recombinant in a wild-type (Leu-683) background afforded the opportunity to compare reversion spectra in the two backgrounds (59) (Fig. 4A versus B). Although replacement of the unacceptable Pro residue by Ser occurs in both cases, the second-site suppressor spectra are entirely different. In the wild-type background only the F700Y suppressor has been obtained (Fig. 4A). The greater size of Tyr and/or the potential for hydrogen bonding involving its phe-

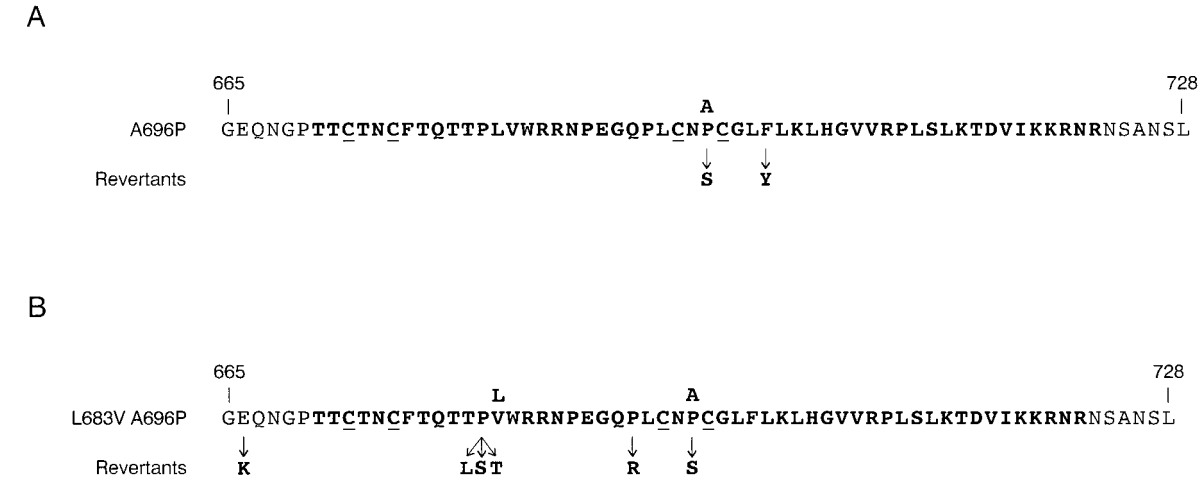

FIG. 4. (A) Revertants of A696P in a wild-type (Leu-683) background (59); (B) revertants of A696P in an L683V background (10, 42). Notation follows that for Fig. 2B.

nolic hydroxyl group might be responsible for its suppressor activity (76). Five different second-site substitutions suppress A696P in a Val-683 background (Fig. 4B). An interesting feature is that the introduction of Pro at position 696 can be compensated by substitutions for Pro at position 682 or 692. This suggests that the structural disruption resulting from the mutant proline residue can be reduced if a nearby proline residue is replaced, presumably restoring greater flexibility and perhaps enabling more contacts to be made (77). The occurrence of the E666K suppressor five residues upstream of the GATA factor homology region (Fig. 4B) is intriguing. Perhaps it increases general DNA affinity by making the environs of the DBD more basic, although it should be noted that it has not appeared as a second-site suppressor in any other cases and that, separated from A696P by fine-structure recombination (Fig. 2), it has no apparent phenotypic effect on nitrogen nutrition. (E666R [Fig. 2B] is similarly without phenotypic effect.)

The P682L suppressor of A696P in an L683V background (Fig. 4B) is particularly noteworthy because P682L is itself a loss-of-function substitution in an otherwise wild-type background (Fig. 1). Such suppression apparently does not occur in a wild-type (Leu-683) background: firstly, efforts to obtain revertants having a mutant AREA sequence from a P682L single-mutation strain have been unsuccessful. More definitively, when a P682L (single-mutation) mutant was crossed to an A696P (single-mutation) mutant, a total of 186 phenotypically wild-type (genotypically confirmed by sequencing in six cases) progeny (resulting from recombination in a 40-bp interval) were recovered on a variety of different selective media without recovery of any putative P682L A696P double mutants (i.e., the reciprocal class) (18). The ability of P682L to suppress A696P in an L683V, but not wild-type, background further underlines the structural, specificity, and physiological consequences of the L683V mutation (7, 14, 31, 37, 42, 65, 77).

Q691P reversion. Q691P is another mutation obtained in an L683V background (40). The result is a pair of adjacent proline residues in β-strand 4 (Fig. 3B), presumably creating steric problems for the β_3-β_4 sheet and eliminating a hydrogen bond contact (76). Substitution of the mutant Pro by a Leu (Fig. 3B) would probably alleviate the steric interference. The N687S suppressor might allow an additional hydrogen bond to compensate that lost due to Q691P (77). The N723K suppressor (Fig. 3B) inserts the lysine present in cGATA-1 at this position (34), where it makes important hydrophobic and hydrogen bonding contacts in the minor groove (55). It is therefore possible that N723K increases the similarity with which the C-terminal tails of the DBDs of AREA and cGATA-1 bind (77).

P709L reversion. The substitution P709L presumably alters the extended loop (residues 706 to 717), perhaps through mispositioning the helical-turn residues (residues 710 to 712), such that one or more important loop residue contacts are no longer possible. It reverts through replacement of the mutant Pro by Ser or by either of two second-site suppressor substitutions (Fig. 3C). The L710P substitution simply reverses the wild-type order of the Pro and Leu residues within the loop, and the V707A suppressor is intriguing in view of the suppression of Q691P in an L683V background by V707M (Fig. 3B).

Second- and Third-Generation Reversions

Highlighting the different modes of AREA interaction with different AREA-controlled promoters and the consequent heterogeneity of promoter responses to any given *areA* mutation, reversion for utilization of one nitrogen source might not improve, and can even further impair, utilization of a second nitrogen source. For example, the P682S L683V A696P and L683V P692R A696P revertants (Fig. 4B), although able to utilize most nitrogen sources quite well, fail to utilize nitrate and nitrite, enabling further reversion, resulting in both cases in replacement of the mutant Val-683 by Met (Fig. 5). However, the second-generation revertants with Met in position 683 are pleiotropically impaired in utilization of other nitrogen sources which their first-generation revertant parents utilize better such as 2-pyrrolidone and L-aspartate. Accordingly, reversion of the second-generation L683M P692R A696P revertant yielded third-generation revertants in one case with a change of residue 683 to Thr and in the other with a change of residue 696 back to the wild-type Ala (Fig. 5B). There is, moreover, no necessity to stop there: the third-generation L683T P692R A696P revertant, although utilizing most nitrogen sources more readily than its second-generation revertant parent, has a diminished appetite for nitrate and uric acid.

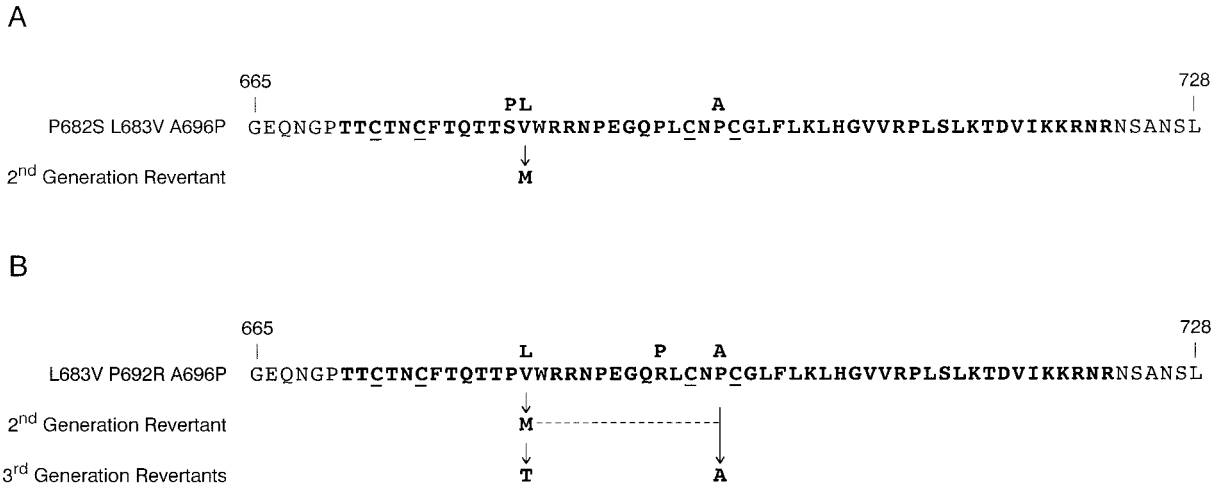

FIG. 5. (A) Second-generation revertant of P682S L683V A696P (10); (B) Second- and third-generation revertants of L683V P692R A696P (10). The third-generation revertants are derived from the second-generation revertant L683M P692R A696P. Notation follows that for Fig. 2B.

Identifying Regions of Significant Tolerance to Sequence Change through Frameshift Reversion

In some cases frameshift reversion yields little or no sequence variation owing to intolerance of the region to sequence change or the nature of the sequence encoded by the alternative reading frames. An example is a −1 frameshift in codon 701 where reversion resulted only in L701W or restoration of the wild-type protein sequence (13, 59). However, in other cases, frameshift reversion reveals a much greater tolerance to sequence change than might have been predicted.

−1 frameshift in codon 688 reversion. Compensating frameshift revertants having at least partial AREA function are shown in Fig. 6. There is considerable tolerance to sequence change in the five-residue region encompassing the C-terminal residue of the β_3 strand through the two N-terminal residues of the β_4 strand. These residues have not been identified as participating in contacts (76). The weakest of the revertants, in which LKE replaces PEGQ, is of particular interest. Firstly, the equivalent of a G690E substitution in the *N. crassa* photoreception- and circadian rhythm-regulating GATA transcription factor WC2 lacks function (49). Secondly, two of its second-generation second-site suppressors (see the legend to Fig. 6) have featured above: F700Y as a suppressor of A696P in a wild-type background (Fig. 4A) and V707M as a suppressor of Q691P in an L683V background (Fig. 3B). Perhaps these changes have rather general effects in improving the stability of AREA-GATA site complexes.

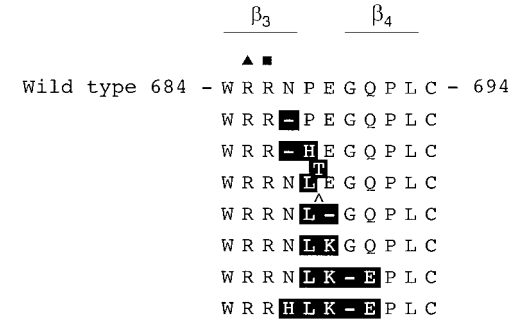

FIG. 6. AREA sequence changes (boxed) resulting from first- and second-generation reversions of a −1 frameshift mutation in codon 688 (84). The bottom sequence is that for a second-generation revertant of the revertant whose sequence is directly above. Other second-generation revertants from the revertant in which LKE replaces PEGQ contain one of the following second-site suppressor substitutions (not shown): F700Y, V707M, or K713R. Structural information at the top is as in Fig. 1.

One further point to note from Fig. 6 is that functional mutant forms of AREA can contain 16 or 18 residues rather than the wild-type 17 in the zinc finger loop. Thus, it is not surprising to find GATA family members whose wild-type forms contain an 18-member zinc finger loop (20, 30, 49, 77a).

−1 frameshift in codon 703 reversion. This frameshift mutation was obtained in an L683V background (18). First- and second-generation revertants are shown in Fig. 7A. These show that, despite the fact that deletion of Lys-702 leads to loss of function (Fig. 1), there is considerable tolerance to sequence change in the C-terminal half of the alpha-helix and N-terminal and central portions of the extended loop, including instances in which substitution and/or deletion affects one or more of six residues involved in major-groove contacts. Remarkably, one of the revertants, albeit able to utilize only a couple of nitrogen sources, contains a deletion of nine contiguous residues in addition to having a Leu→Phe substitution. Some positions involved in second-generation second-site suppression (see the legend to Fig. 7) have featured previously. A Met residue at position 683 resulted from second-generation reversion of A696P in an L683V background (Fig. 5). Met in position 683 alters specificity of gene expression (14, 42, 45, 65) and probably increases the degree to which binding to CGATA sites is favored over binding to TGATA sites (65). K713R was also obtained as a second-site suppressor of the mutant in which LKE replaces PEGQ (see the legend to Fig. 6) suggesting that increasing the side chain length, basicity, and/or hydrogen bonding potential at this position increases DNA binding affinity. The F700Y change, obtained here as a second-generation suppressor, features for the third time, suggesting that it might have a very general effect in improving DNA binding.

The compensating frameshift mutations have in some cases been separated from the L683V mutation by recombination using crosses to a strain carrying a suitably positioned loss-of-function *areA* mutation (Fig. 7B). The L683V phenotype tends to be approximately additive with those of the changes in the C-terminal part of the alpha-helix. Separation of the compensating frameshifts from the L683V mutation is critically conti-

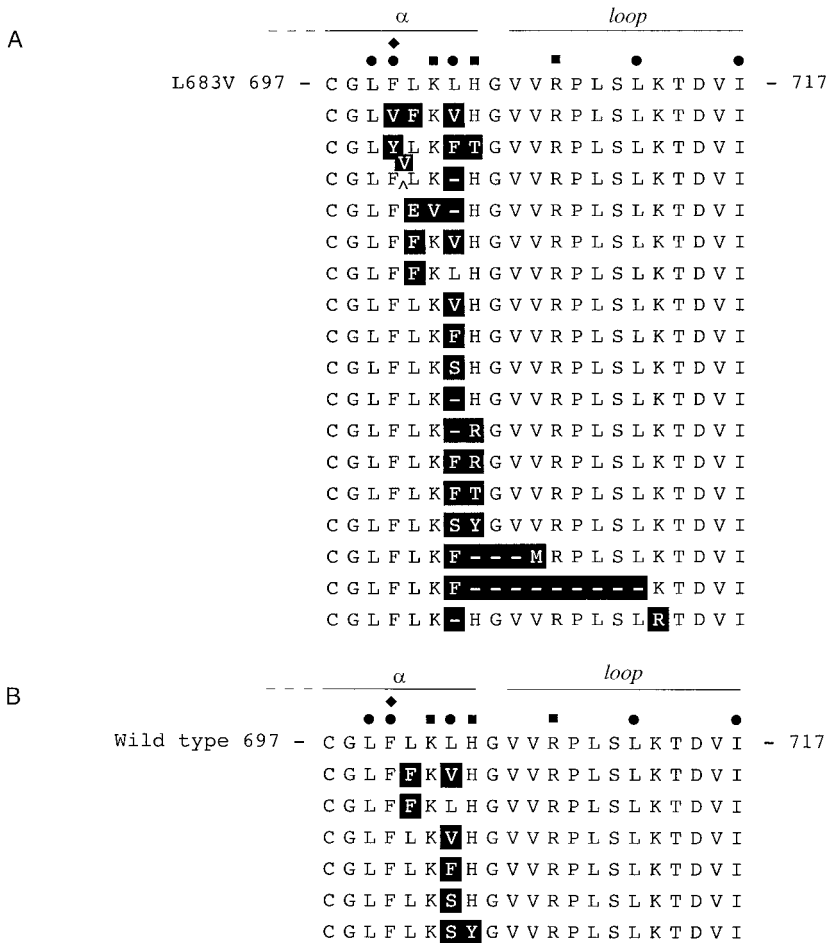

FIG. 7. (A) AREA sequence changes resulting from first- and second-generation reversions of a −1 frameshift mutation in codon 703 in an L683V background (84), displayed as in Fig. 6. Second-generation revertants of the L683V ΔL703 mutant are the L683V 700+V ΔL703, L683V ΔL703 H704R, and L683V ΔL703 K713R mutants plus the duplication mutant described in the text. Second-generation revertants of the L683V L701F L703V mutant are the L683V L701F, L683V F700V L701F L703V, N675Y L683V L701F L703V (not shown), and L683M L701F L703V (not shown) mutants. Second-generation revertants of L683V L703F H704T are the L683V F700Y L703F H704T and L683V L703F H704R mutants. N675I L683V L703V (not shown) is a second-generation revertant of L683V L703V. (B) Mutational changes (functional) in panel A separated from L683V (and therefore in a Leu-683 background) by genetic recombination (84), displayed as in Fig. 6. An N675I L703V double mutant was identified among progeny of a cross designed to separate N675I from L683V and L703V in the second-generation revertant N675I L683V L703V. It must have arisen either by a double-recombination event (involving intervals of 21 and 58 bp) or by reversion of the mutation responsible for the L683V change. The lack of recovery of the single change N675I, by what should have been a more frequent recombination event, suggests that N675I might result in a nonfunctional AREA protein, particularly as we have also been unable to obtain an N675I recombinant by outcrossing an N675I L703V strain in which the (single) relevant recombination interval would be 80 bp.

gent upon identifying a nitrogen source which strains carrying the L683V mutation (with or without the compensating frameshifts) cannot utilize but which recombinants having the compensating frameshifts in a wild-type (Leu-683) background can utilize.

Increasing Sequence Similarity to Other GATA Factors

The P682S, P682T, N687S, F700Y, L701F, V707M, K713R, and N723K second-site suppressor substitutions are all notable for resulting in a residue which occurs in the equivalent position in at least one other GATA factor (32, 34, 39, 47, 53, 73, 89). This underlines both the functional similarity and the homology of the DBDs of the GATA family of transcription factors.

Duplicating the DBD

A mutation selected as elevating AREA function with respect to pyrimidine salvage results in tandem duplication of (wild-type) residues 658 to 796, which include the DBD (22, 68). Among the second-generation revertants of the L683V ΔL703 first-generation revertant (Fig. 7A) was one resulting in a tandem duplication of residues 662 to 783 (with L683V ΔL703 in both copies), also encompassing the DBD (84). In at least the former case, there is evidence that the two copies of the DBD act independently and alternately rather than jointly (22).

It thus appears that one way of elevating AREA function is through duplication of the DBD, whether wild type or mutant. Perhaps the presence of two neighboring copies of the DBD enhances the likelihood that dissociation of the DBD from a GATA site will be followed by reassociation and/or the speed with which such reassociation occurs. Mutational duplication of the DBD is probably also of evolutionary relevance, providing a modern model for an ancient event in the evolution of GATA family members containing two copies of the region of GATA factor homology (22). It is worth noting that the (derived) primary-structure distance between the 52-residue

GATA factor homology regions is much greater in the two *areA* duplication mutants (87 and 70 amino acids, respectively) than in vertebrate GATA factors (only 2 amino acids in murine GATA-1 [mGATA-1] [79], for example) but much more similar to those in fungal GATA factors having two copies of the GATA region such as *U. maydis* Urbs1 and its *Penicillium chrysogenum* homolog SREP (92 amino acids in each case) (38, 80). Phylogenetic trees of GATA factors have been constructed (66, 77a).

USE OF THE AREA SYSTEM TO ANALYZE THE DBD OF A VERTEBRATE GATA FACTOR

Caddick et al. (24) have shown that the AREA system of *A. nidulans* can be utilized to analyze the DBDs of other GATA factors, including those which contain two copies of the GATA homology domain. Using a null *areA* mutant as transformation recipient, they firstly showed that mGATA-1 is able to substitute AREA for the utilization of a number of nitrogen sources, the effectiveness of the substitution apparently being dependent on the copy number and genome position of the mGATA-1 sequence(s). They then analyzed a number of chimeric constructs in which the mGATA-1 N-terminal and/or C-terminal GATA domain(s) in various positions would either replace or occur in addition to the AREA GATA domain. The chimera in which the entire double GATA domain of mGATA-1 replaced the GATA domain of AREA was characterized genetically. Interestingly, although replacement of the AREA GATA domain by the C-terminal mGATA-1 GATA domain was very functional, the additional presence of the N-terminal GATA domain of mGATA-1 made it much less so (24). Mutations in the chimeric gene impairing the N-terminal GATA domain strongly increase functionality (24). A chimera containing the N-terminal GATA domain of mGATA-1 alone is unable to substitute AREA (24).

PERSPECTIVES

We hope that this survey of sequence changes in the AREA DBD will aid those working with other transcription factors, particularly other GATA family members less amenable to genetic analysis. Where corresponding sequence changes have been characterized in other GATA factor DBDs, their phenotypes with regard to functionality agree with those found for AREA. The surprising degree of tolerance of certain regions of the AREA DBD to sequence change should also not escape attention. One general point from studies of AREA particularly deserves re-emphasizing: any transcription factor which interacts with more than one promoter is likely to differ, at least to some extent, in the way it interacts at different promoters. It follows that monitoring the behavior of one or even several structural genes is insufficient to characterize adequately mutant forms of the transcription factor and might even be misleading. It also follows that descriptions useful for many structural-gene mutations such as thermosensitive, osmotic remedial, leaky, and cryosensitive are not applicable to mutations in most genes encoding transcription factors unless the promoter(s) at which they apply is also specified. There is no reason to believe that *areA* has a monopoly among genes encoding transcription factors on the aspect of having an astonishing range of mutant phenotypes.

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REFERENCES

1. **Abel, T., A. M. Michelson, and T. Maniatis.** 1993. A *Drosophila* GATA family member that binds to *Adh* regulatory sequences is expressed in the developing fat body. Development **119:**623–633.
2. **Al Taho, N. M.** 1982. Suppressible alleles in the *areA* gene of *Aspergillus nidulans*. M.Sc. thesis. University of Essex, Colchester, United Kingdom.
3. **Al Taho, N. M., H. M. Sealy-Lewis, and C. Scazzocchio.** 1984. Suppressible alleles in a wide domain regulatory gene in *Aspergillus nidulans*. Curr. Genet. **8:**245–251.
4. **An, Z., Q. Zhao, J. McEvoy, W. M. Yuan, J. L. Markley, and S. A. Leong.** 1997. The second finger of Urbs1 is required for iron-mediated repression of *sid1* in *Ustilago maydis*. Proc. Natl. Acad. Sci. USA **94:**5882–5887.

4a. **Andrianopoulos, A., S. Kourambas, J. A. Sharp, M. A. Davis, and M. J. Hynes.** 1998. Characterization of the *Aspergillus nidulans nmrA* gene involved in nitrogen metabolite repression. J. Bacteriol. **180:**1973–1977.

5. **Arst, H. N., Jr.** 1989. The *areA* gene mediating nitrogen metabolite repression in *Aspergillus nidulans*, p. 53–62. *In* H. Nevalainen and M. Penttilä (ed.), Molecular biology of filamentous fungi. Foundation for Biotechnical and Industrial Fermentation Research, Helsinki, Finland.
6. **Arst, H. N., Jr., and C. R. Bailey.** 1977. The regulation of carbon metabolism, p. 131–145. *In* J. E. Smith and J. A. Pateman (ed.), Genetics and physiology of *Aspergillus*. Academic Press, London, United Kingdom.
7. **Arst, H. N., Jr., and D. J. Cove.** 1973. Nitrogen metabolite repression in *Aspergillus nidulans*. Mol. Gen. Genet. **126:**111–141.
8. **Arst, H. N., Jr., S. A. Jones, and C. R. Bailey.** 1981. A method for the selection of deletion mutations in the L-proline catabolism gene cluster of *Aspergillus nidulans*. Genet. Res. **38:**171–195.
9. **Arst, H. N., Jr., B. Kudla, N. Martinez-Rossi, M. X. Caddick, S. Sibley, and R. W. Davies.** 1989. *Aspergillus* and mouse share a new class of 'zinc finger' protein. Trends Genet. **5:**291.
10. **Arst, H. N., Jr., and T. Langdon.** Unpublished data.
11. **Arst, H. N., Jr., and D. W. MacDonald.** 1975. A gene cluster in *Aspergillus nidulans* with an internally located *cis*-acting regulatory region. Nature (London) **254:**26–31.
12. **Arst, H. N., Jr., D. W. MacDonald, and S. A. Jones.** 1980. Regulation of proline transport in *Aspergillus nidulans*. J. Gen. Microbiol. **116:**285–294.
13. **Arst, H. N., Jr., and J. M. Mates-Sanchez.** Unpublished data.
14. **Arst, H. N., Jr., and C. Scazzocchio.** 1975. Initiator constitutive mutation with an 'up-promoter' effect in *Aspergillus nidulans*. Nature (London) **254:**31–34.
15. **Arst, H. N., Jr., and C. Scazzocchio.** 1985. Formal genetics and molecular biology of the control of gene expression in *Aspergillus nidulans*, p. 309–343. *In* J. W. Bennett and L. L. Lasure (ed.), Gene manipulations in fungi. Academic Press, Inc., Orlando, Fla.
16. **Arst, H. N., Jr., and A. Sheerins.** 1996. Translational initiation competence, 'leaky scanning' and translational reinitiation in *areA* mRNA of *Aspergillus nidulans*. Mol. Microbiol. **19:**1019–1024.
17. **Arst, H. N., Jr., D. Tollervey, and M. X. Caddick.** 1989. A translocation-associated loss of function mutation in the nitrogen metabolite repression gene of *Aspergillus nidulans* can revert intracistronically. Mol. Gen. Genet. **215:**364–367.
18. **Arst, H. N., Jr., and R. A. Wilson.** Unpublished data.
19. **Arst, H. N., Jr., R. A. Wilson, and T. Langdon.** Unpublished data.

19a. **Ballario, P., and G. Macino.** 1997. White collar proteins: PASsing the light signal in *Neurospora crassa*. Trends Microbiol. **5:**458–462.

20. **Ballario, P., P. Vittorioso, A. Magrelli, C. Talora, A. Cabibbo, and G. Macino.** 1996. White collar-1, a central regulator of blue light responses in *Neurospora*, is a zinc finger protein. EMBO J. **15:**1650–1657.
21. **Bobola, N., R.-P. Jansen, T. H. Shin, and K. Nasmyth.** 1996. Asymmetric accumulation of Ash1p in postanaphase nuclei depends on a myosin and restricts yeast mating-type switching to mother cells. Cell **84:**699–709.
22. **Caddick, M. X., and H. N. Arst, Jr.** 1990. Nitrogen regulation in *Aspergillus*: are two fingers better than one? Gene **95:**123–127.
23. **Caddick, M. X., D. Peters, and A. Platt.** 1994. Nitrogen regulation in fungi. Antonie Leeuwenhoek **65:**169–177.
24. **Caddick, M. X., D. G. Peters, P. Hooley, and A. Nayler.** 1997. Genetic and molecular characterization of murine GATA-1 in *Aspergillus* defines a critical role for the N-terminal finger. Genes Funct. **1:**37–49.

24a. **Chandarlapaty, S., and B. Errede.** 1998. Ash1, a daughter cell-specific protein, is required for pseudohyphal growth of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **18:**2884–2891.

25. **Chang, W.-T., P. C. Newell, and J. D. Gross.** 1996. Identification of the cell fate gene *stalky* in Dictyostelium. Cell **87:**471–481.
26. **Coffman, J. A., R. Rai, T. Cunningham, V. Svetlov, and T. G. Cooper.** 1996. Gat1p, a GATA family protein whose production is sensitive to nitrogen catabolite repression, participates in transcriptional activation of nitrogen-catabolic genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:**847–858.

27. **Crawford, N. M., and H. N. Arst, Jr.** 1993. The molecular genetics of nitrate assimilation in fungi and plants. Annu. Rev. Genet. **27:**115–146.
28. **Crosthwaite, S. K., J. C. Dunlop, and J. J. Loros.** 1997. *Neurospora wc-1* and *wc-2*: transcription, photoresponses, and the origins of circadian rhythmicity. Science **276:**763–769.
29. **Cubero, B., and C. Scazzocchio.** 1994. Two different adjacent and divergent zinc finger binding sites are necessary for CREA-mediated carbon catabolite repression in the proline gene cluster of *Aspergillus nidulans*. EMBO J. **13:** 407–415.
30. **Daniel-Vedele, F., and M. Caboche.** 1993. A tobacco cDNA clone encoding a GATA-1 zinc finger protein homologous to regulators of nitrogen metabolism in fungi. Mol. Gen. Genet. **240:**365–373.
31. **Diallinas, G., L. Gorfinkiel, H. N. Arst, Jr., G. Cecchetto, and C. Scazzocchio.** 1995. Genetic and molecular characterization of a gene encoding a wide specificity purine permease of *Aspergillus nidulans* reveals a novel family of transporters conserved in prokaryotes and eukaryotes. J. Biol. Chem. **270:**8610–8622.
32. **Drevet, J. R., Y. A. W. Sheiky, and K. Iatrou.** 1994. GATA-type zinc finger motif-containing sequences and chorion gene transcription factors of the silkworm *Bombyx mori*. J. Biol. Chem. **269:**10660–10667.
33. **Drevet, J. R., L. Swevers, and K. Iatrou.** 1995. Developmental regulation of a silkworm gene encoding multiple GATA-type transcription factors by alternative splicing. J. Mol. Biol. **246:**43–53.
34. **Evans, T., and G. Felsenfield.** 1989. The erythroid-specific transcription factor Eryf1: a new finger protein. Cell **58:**877–885.
35. **Fu, Y.-H., and G. A. Marzluf.** 1990. *nit-2*, the major positive-acting nitrogen regulatory gene of *Neurospora crassa*, encodes a sequence-specific DNA-binding protein. Proc. Natl. Acad. Sci. USA **87:**5331–5335.
36. **Fu, Y. H., and G. A. Marzluf.** 1990. Site-directed mutagenesis of the 'zinc finger' DNA-binding domain of the nitrogen-regulatory protein NIT2 of *Neurospora*. Mol. Microbiol. **4:**1847–1852.
37. **Gorfinkiel, L., G. Diallinas, and C. Scazzocchio.** 1993. Sequence and regulation of the *uapA* gene encoding a uric acid-xanthine permease in the fungus *Aspergillus nidulans*. J. Biol. Chem. **268:**23376–23381.
38. **Haas, H., K. Angermayr, and G. Stöffler.** 1997. Molecular analysis of a *Penicillium chrysogenum* GATA factor encoding gene (*sreP*) exhibiting significant homology to the *Ustilago maydis urbs1* gene. Gene **184:**33–37.
39. **Hawkins, M. G., and J. D. McGhee.** 1995. *elt-2*, a second GATA factor from the nematode *Caenorhabditis elegans*. J. Biol. Chem. **270:**14666–14671.
40. **Hynes, M. J.** 1972. Mutants with altered glucose repression of amidase enzymes in *Aspergillus nidulans*. J. Bacteriol. **111:**717–722.
41. **Hynes, M. J.** 1973. Pleiotropic mutants affecting the control of nitrogen metabolism in *Aspergillus nidulans*. Mol. Gen. Genet. **125:**99–107.
42. **Hynes, M. J.** 1975. Studies on the role of the *areA* gene in the regulation of nitrogen catabolism in *Aspergillus nidulans*. Aust. J. Biol. Sci. **28:**301–313.
43. **Jones, S. A., H. N. Arst, Jr., and D. W. MacDonald.** 1981. Gene roles in the *prn* cluster of *Aspergillus nidulans*. Curr. Genet. **3:**49–56.
44. **Ko, L. J., and J. D. Engel.** 1993. DNA-binding specificities of the GATA transcription factor family. Mol. Cell. Biol. **13:**4011–4022.
45. **Kudla, B., M. X. Caddick, T. Langdon, N. M. Martinez-Rossi, C. F. Bennett, S. Sibley, R. W. Davies, and H. N. Arst, Jr.** 1990. The regulatory gene *areA* mediating nitrogen metabolite repression in *Aspergillus nidulans*. Mutations affecting specificity of gene activation alter a loop residue of a putative zinc finger. EMBO J. **9:**1355–1364.
46. **Langdon, T., A. Sheerins, A. Ravagnani, M. Gielkens, M. X. Caddick, and H. N. Arst, Jr.** 1995. Mutational analysis reveals dispensability of the N-terminal region of the *Aspergillus* transcription factor mediating nitrogen metabolite repression. Mol. Microbiol. **17:**877–888.
47. **Lee, M.-E., D. H. Temizer, J. A. Clifford, and T. Quertermous.** 1991. Cloning of the GATA-binding protein that regulates endothelin-1 gene expression in endothelial cells. J. Biol. Chem. **266:**16188–16192.
48. **Lin, W.-H., L.-H. Huang, J.-Y. Yeh, J. Hoheisel, H. Lehrach, Y. H. Sun, and S.-F. Tsai.** 1995. Expression of a *Drosophila* GATA transcription factor in multiple tissues in the developing embryos. Identification of homozygous lethal mutants with P-element insertion at the promoter region. J. Biol. Chem. **270:**25150–25158.
49. **Linden, H., and G. Macino.** 1997. White collar 2, a partner in blue light signal transduction, controlling expression of light-regulated genes in *Neurospora crassa*. EMBO J. **16:**98–109.
50. **Mackay, J. P., and M. Crossley.** 1998. Zinc fingers are sticking together. Trends Biochem. Sci. **23:**1–4.

50a. **Martin, D. I. K., and S. H. Orkin.** 1990. Transcriptional activation and DNA binding by the erythroid factor GF-1/NF-E1/Eryf 1. Genes Dev. **4:**1886–1898.

51. **Marzluf, G. A.** 1997. Genetic regulation of nitrogen metabolism in the fungi. Microbiol. Mol. Biol. Rev. **61:**17–32.
52. **Merika, M., and S. H. Orkin.** 1993. DNA-binding specificity of GATA family transcription factors. Mol. Cell. Biol. **13:**3999–4010.
53. **Minehart, P. L., and B. Magasanik.** 1991. Sequence and expression of *GLN3*, a positive nitrogen regulatory gene of *Saccharomyces cerevisiae* encoding a protein with a putative zinc finger DNA-binding domain. Mol. Cell. Biol. **11:**6216–6228.
54. **Oestreicher, N., C. Scazzocchio, and T. Suárez.** 1997. Mutations in a dispensable region of the UaY transcription factor of *Aspergillus nidulans* differentially affect the expression of structural genes. Mol. Microbiol. **24:**1189–1199.
55. **Omichinski, J. G., G. M. Clore, O. Schaad, G. Felsenfeld, C. Trainor, E. Appella, S. J. Stahl, and A. M. Gronenborn.** 1993. NMR structure of a specific DNA complex of Zn-containing DNA binding domain of GATA-1. Science **261:**438–446.
56. **Pan, H., B. Feng, and G. A. Marzluf.** 1997. Two distinct protein-protein interactions between the NIT2 and NMR regulatory proteins are required to establish nitrogen metabolite repression in *Neurospora crassa*. Mol. Microbiol. **26:**721–729.
57. **Pedone, P. V., J. G. Omichinski, P. Nony, C. Trainor, A. M. Gronenborn, G. M. Clore, and G. Felsenfeld.** 1997. The N-terminal fingers of chicken GATA-2 and GATA-3 are independent sequence-specific DNA binding domains. EMBO J. **16:**2874–2882.
58. **Peters, D. G., and M. X. Caddick.** 1994. Direct analysis of native and chimeric GATA specific DNA binding proteins from *Aspergillus nidulans*. Nucleic Acids Res. **22:**5164–5172.
59. **Platt, A., T. Langdon, H. N. Arst, Jr., D. Kirk, D. Tollervey, J. M. Mates Sanchez, and M. X. Caddick.** 1996. Nitrogen signalling involves the C-terminus and the GATA domain of the *Aspergillus* transcription factor AREA and the 3′ untranslated region of its mRNA. EMBO J. **15:**2791–2801.
60. **Platt, A., A. Ravagnani, H. Arst, Jr., D. Kirk, T. Langdon, and M. X. Caddick.** 1996. Mutational analysis of the C-terminal region of AREA, the transcription factor mediating nitrogen metabolite repression in *Aspergillus nidulans*. Mol. Gen. Genet. **250:**106–114.
61. **Ramain, P., P. Heitzler, M. Haenlin, and P. Simpson.** 1993. *pannier*, a negative regulator of *achaete* and *scute* in *Drosophila*, encodes a zinc finger protein with homology to the vertebrate transcription factor GATA-1. Development **119:**1277–1291.
62. **Rand, K. N.** 1978. Aspects of the control of nitrogen metabolism in *Aspergillus nidulans*. Ph.D. thesis. University of Cambridge, Cambridge, United Kingdom.
63. **Rand, K. N., and H. N. Arst, Jr.** 1977. A mutation in *Aspergillus nidulans* which affects the regulation of nitrite reductase and is tightly linked to its structural gene. Mol. Gen. Genet. **155:**67–75.
64. **Ravagnani, A., and H. N. Arst, Jr.** 1995. Quantitation of oligohistidine fusion proteins using $^{63}Ni^{2+}$ chelation. Nucleic Acids Res. **23:**3069–3070.
65. **Ravagnani, A., L. Gorfinkiel, T. Langdon, G. Diallinas, E. Adjadj, S. Demais, D. Gorton, H. N. Arst, Jr., and C. Scazzocchio.** 1997. Subtle hydrophobic interactions between the seventh residue of the zinc finger loop and the first base of an HGATAR sequence determine promoter-specific recognition by the *Aspergillus nidulans* GATA factor AreA. EMBO J. **16:**3974–3986.
66. **Rehorn, K.-P., H. Thelen, A. M. Michelson, and R. Reuter.** 1996. A molecular aspect of hematopoiesis and ectoderm development common to vertebrates and *Drosophila*. Development **122:**4023–4031.
67. **Scazzocchio, C.** Personal communication.
68. **Shaffer, P. M., and H. N. Arst, Jr.** 1984. Regulation of pyrimidine salvage in *Aspergillus nidulans*: a role for the major regulatory gene *areA* mediating nitrogen metabolite repression. Mol. Gen. Genet. **198:**139–145.
69. **Sil, A., and I. Herskowitz.** 1996. Identification of an asymmetrically localized determinant, Ash1p, required for lineage-specific transcription of the yeast HO gene. Cell **84:**711–722.
70. **Simon, M. C.** 1995. Gotta have GATA. Nat. Genet. **11:**9–11.
71. **Sophianopoulou, V., T. Suárez, G. Diallinas, and C. Scazzocchio.** 1993. Operator depressed mutations in the proline utilisation gene cluster of *Aspergillus nidulans*. Mol. Gen. Genet. **236:**209–213.
72. **Soussi-Boudekou, S., S. Vissers, A. Urrestarazu, J.-C. Jauniaux, and B. André.** 1997. Gzf3p, a fourth GATA factor involved in nitrogen-regulated transcription in *Saccharomyces cerevisiae*. Mol. Microbiol. **23:**1157–1168.
73. **Spieth, J., Y.-H. Shim, K. Lea, R. Conrad, and T. Blumenthal.** 1991. *elt-1*, an embryonically expressed *Caenorhabditis elegans* gene homologous to the GATA transcription factor family. Mol. Cell. Biol. **11:**4651–4659.
74. **Stanbrough, M., D. W. Rowen, and B. Magasanik.** 1995. Role of GATA factors Gln3p and Nil1p of *Saccharomyces cerevisiae* in the expression of nitrogen-regulated genes. Proc. Natl. Acad. Sci. USA **92:**9450–9454.
75. **Stankovich, M., A. Platt, M. X. Caddick, T. Langdon, P. M. Shaffer, and H. N. Arst, Jr.** 1993. C-terminal truncation of the transcriptional activator encoded by *areA* in *Aspergillus nidulans* results in both loss-of-function and gain-of-function phenotypes. Mol. Microbiol. **7:**81–87.
76. **Starich, M. R., M. Wikström, H. N. Arst, Jr., G. M. Clore, and A. M. Gronenborn.** 1998. The solution structure of a fungal AREA protein-DNA complex: an alternative binding mode for the basic carboxyl tail of GATA factors. J. Mol. Biol. **277:**605–620.
77. **Starich, M. R., M. Wikström, S. Schumacher, H. N. Arst, Jr., A. M. Gronenborn, and G. M. Clore.** 1998. The solution structure of the Leu22→Val mutant AREA DNA binding domain complexed with a TGATAG core element defines a role for hydrophobic packing in the determination of specificity. J. Mol. Biol. **277:**621–634.

77a. **Teakle, G. R., and P. M. Gilmartin.** 1998. Two forms of zinc-finger motif and

their kingdom-specific distribution between the flora, fauna and fungi. Trends Biochem. Sci. **23:**100–102.
78. **Tollervey, D. W.** 1981. Aspects of nitrogen metabolic regulation in *Aspergillus nidulans*. Ph.D. thesis. University of Cambridge, Cambridge, United Kingdom.
79. **Tsai, S.-F., D. I. K. Martin, L. I. Zon, A. D. D'Andrea, G. G. Wong, and S. H. Orkin.** 1989. Cloning of cDNA for the major DNA-binding protein of the erythroid lineage through expression in mammalian cells. Nature (London) **339:**446–451.
80. **Voisard, C., J. Wang, J. L. McEvoy, P. Xu, and S. A. Leong.** 1993. *urbs1*, a gene regulating siderophore biosynthesis in *Ustilago maydis*, encodes a protein similar to the erythroid transcription factor GATA-1. Mol. Cell. Biol. **13:** 7091–7100.
81. **Weiss, M. J., and S. H. Orkin.** 1995. GATA transcription factors: key regulators of hematopoiesis. Exp. Hematol. **23:**99–107.
81a. **Whyatt, D. J., E. deBoer, and F. Grosveld.** 1993. The two zinc finger-like domains of GATA-1 have different DNA binding specificities. EMBO J. **12:** 4993–5005.
82. **Whyatt, D. J., A. Karis, I. C. Harkes, A. Verkerk, N. Gillemans, A. G. Elefanty, G. Vairo, R. Ploemacher, F. Grosveld, and S. Philipsen.** 1997. The level of the tissue-specific factor GATA-1 affects the cell-cycle machinery. Genes Funct. **1:**11–24.
83. **Wiame, J.-M., M. Grenson, and H. N. Arst, Jr.** 1985. Nitrogen catabolite repression in yeasts and filamentous fungi. Adv. Microb. Physiol. **26:**1–88.
84. **Wilson, R. A., and H. N. Arst, Jr.** Unpublished data.
85. **Wilson, R. A., H. N. Arst, Jr., and T. Langdon.** Unpublished data.
86. **Wilson, R. A., H. N. Arst, Jr., S. D. Polley, and M. X. Caddick.** Unpublished data.
87. **Xiao, X., Y.-H. Fu, and G. A. Marzluf.** 1995. The negative-acting NMR regulatory protein of *Neurospora crassa* binds to and inhibits the DNA-binding activity of the positive-acting nitrogen regulatory protein NIT2. Biochemistry **34:**8861–8868.
88. **Xiao, X., and G. A. Marzluf.** 1993. Amino-acid substitutions in the zinc finger of NIT2, the nitrogen regulatory protein of *Neurospora crassa*, alter promoter element recognition. Curr. Genet. **24:**212–218.
89. **Yamamoto, M., L. J. Ko, M. W. Leonard, H. Beug, S. H. Orkin, and J. D. Engel.** 1990. Activity and tissue-specific expression of the transcription factor NF-E1 multigene family. Genes Dev. **4:**1650–1662.
90. **Yang, H.-Y., and T. Evans.** 1992. Distinct roles for the two cGATA-1 finger domains. Mol. Cell. Biol. **12:**4562–4570.